

# Early Contributions to Infants' Mental Rotation Abilities

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### ***Research Highlights***

- This is the first study to provide evidence of a correlation between testosterone concentrations during the early postnatal testosterone surge (“mini-puberty”) and performance on a mental rotation task in boys at 5 – 6 months of age.
- The study is also the first to provide evidence of a relationship between parental attitudes about gender and mental rotation performance in girls at 5 – 6 months of age.
- The study is the first to provide evidence of an early postnatal surge in salivary testosterone. Previously, this surge has been seen in serum and urine samples, but not in saliva samples. Our data suggest that the surge can also be detected using saliva samples in infants at 1 – 2.5 months of age.
- This study reports a moderate-to-large gender difference in infants’ mental rotation abilities at 5 – 6 months of age, with male infants more likely to show mental rotation ability than female infants.

***Abstract***

Some cognitive abilities exhibit reliable gender differences, with females outperforming males in specific aspects of verbal ability, and males showing an advantage on certain spatial tasks. Among these cognitive gender differences, differences in mental rotation are the most robust, and appear to be present even in infants. A large body of animal research suggests that gonadal hormones, particularly testosterone, during early development could contribute to this gender difference in mental rotation. Also, substantial evidence supports an influence of socialization on mental rotation performance. The present study investigated the relationship of two types of factors, early postnatal testosterone exposure and parental attitudes about gender, to mental rotation performance in 61 healthy infants (29 males, 32 females). We measured salivary testosterone at two time points: 1 – 2.5 months of age and 5 – 6 months of age. Infants' mental rotation performance and parents' attitudes about gender were assessed at 5 – 6 months of age. As predicted, testosterone concentrations were significantly higher in boys than girls in early infancy ( $d = 0.54$ ), and boys performed significantly better than girls on mental rotation ( $d = 0.64$ ). A significant positive correlation between testosterone at age 1 - 2.5 months and mental rotation was found only in boys ( $r = 0.50, p = 0.01$ ). A significant negative correlation between parents' gender-stereotypical attitudes and mental rotation performance was found only in girls ( $r = -.57, p = .002$ ). These findings suggest that the early postnatal testosterone surge (also known as “mini-puberty”) may have organizational influences on mental rotation performance in boys, and that parents may influence their daughters' mental rotation abilities beginning very early in life.

***Keywords:*** *Mental rotation; Testosterone; Gender; Infancy; Parental socialization.*

Some cognitive abilities differ on average for males and females. Notably, females outperform males on average on some measures of verbal ability, while males outperform females on average on some measures of visuo-spatial abilities (Halpern, 2000). Mental rotation — i.e., the ability to transform a mental representation of an object so as to accurately predict how the object would look from a different angle (Shepard & Metzler, 1971) — has been a key area in gender differences research over the last few decades, perhaps because the gender difference in mental rotation abilities is among the most robust and stable of cognitive gender differences, with meta-analyses showing moderate to large effect sizes ( $d = 0.73$ , Linn & Petersen, 1985;  $d = 0.56$ , Voyer et al., 1995) across different age groups from childhood into adulthood.

The large and persistent gender difference in mental rotation performance is potentially significant for gender differences in other areas. For instance, mental rotation abilities are believed to play an important role in scientific and creative professions. Mental rotation also has been related anecdotally to scientific breakthroughs, such as Watson and Crick's discovery of the structure of DNA, Tesla's vision of rotating magnetic fields, and Einstein's theory of relativity (Shepard & Cooper, 1986). Similarly, mental rotation abilities have been suggested to be important for various professional careers like architecture, engineering, navigation, and medicine (Kerkman et al., 2000; Uttal & Cohen, 2012). Good spatial abilities early in life have also been found to predict subsequent success in STEM subjects (Shea et al., 2001; Wai et al., 2009). More mundanely, mental rotation abilities have been linked to better performance in spatial orientation tasks such as navigating the geography of a new city or finding one's car in the parking lot when approaching from a new direction (Levine et al., 1999).

The underlying causes of the gender difference in mental rotation are not yet fully understood. Most gender differences in human behavior result from numerous factors interacting over time. Specific factors that appear to play a role in gender development broadly include exposure to gonadal steroids — particularly testosterone — during early development (prenatally/neonatally), as well as postnatal socialization by parents, teachers, peers and others, and self-socialization based on cognitive understanding of gender (Hines, 2015). The contribution of each type of factor may differ, however, for different gender-related outcomes. For instance, numerous studies suggest that children's toy preferences are influenced by testosterone exposure during prenatal, and perhaps neonatal, periods of early development, when testosterone is higher in developing males than females (Constantinescu & Hines, 2012). In contrast, evidence for similar hormonal influences on gender differences in cognitive performance is less consistent (Hines, 2015), and the sizes of gender differences in some cognitive abilities (e.g., reading and math) appear to vary culturally in ways that suggest powerful social influences (Guiso et al., 2008; Kane & Mertz, 2012).

Regarding mental rotation performance specifically, the gender difference seems to appear very early in life. Of the eight studies published so far that report mental rotation abilities in early infancy (Hespos & Rochat, 1997; Möhring & Frick, 2013; Moore & Johnson, 2008, 2011; Quinn & Liben, 2008, 2014; Rochat & Hespos, 1996; Schwarzer, Freitag, & Schum, 2013), four have found gender differences favouring males as early as 3 – 9 months of age (Moore & Johnson, 2008, 2011; Quinn & Liben, 2008, 2014). (Möhring & Frick, 2013 and Schwarzer, Freitag, & Schum, 2013 analyzed for gender differences and reported none; Hespos & Rochat, 1997 and Rochat & Hespos, 1996 did not report analyses of gender differences.) Furthermore, although many cognitive gender differences appear to have decreased during the last five decades (Feingold, 1988; Hyde et al., 1990), perhaps in

response to social change (Hyde, 2005; Spelke, 2005), the size of the gender difference in mental rotation abilities appears to have remained large and stable (Masters & Sanders, 1993; Voyer et al., 1995). The possible emergence of a gender difference in mental rotation in infancy, and its stability over the last several decades, might suggest that the gender difference relates in part to early emerging influences, such as exposure to testosterone during early development.

### **Early Testosterone Exposure and Human Gender-Related Behavior**

A large body of experimental research in non-human mammals suggests that testosterone exposure during early periods of rapid brain development has enduring influences on the brain and on behavior (Arnold, 2009; Hines, 2015). In humans as well, many gender differences in behavior/psychological characteristics have been studied in relation to early testosterone exposure. To discuss these influences, it will be helpful to provide more information on the times when testosterone is likely to influence human gender development, to outline the sizes of gender differences in human behavior, and to describe the approaches used to assess possible influences of testosterone during early development on human behavior.

#### **When would testosterone be hypothesized to influence human gender development?**

Thousands of studies have been conducted to identify the mechanisms involved in sexual differentiation of the mammalian brain and behavior (Arnold, 2009). These studies suggest that during early development, testosterone has enduring influences on later behavior, apparently because this gonadal steroid influences processes of brain organization. For instance, during perinatal development, testosterone and its metabolites influence neuronal

survival and growth, including the growth of axons and dendrites, and influence the eventual size and connectivity of some brain regions (McCarthy et al., 2009). The enduring effects of testosterone early in life on later behavior are therefore thought to reflect early, enduring changes in brain organization, and have been termed *organizational effects*. These contrast with the transient effects that gonadal steroids can have on behavior later in life, effects that are present when the hormone is present, but not after it is gone. In contrast to organizational effects, the transient effects of gonadal hormones on behavior have been termed *activational effects*.

The large body of research in non-human mammals also suggests that the organizational influences of testosterone on later behavior occur during perinatal periods when testosterone is higher in developing males than females. Depending on the species, these periods can be prenatal, neonatal, or both. During human development, testosterone is higher in male than in female fetuses from about week 8 to week 24 of gestation (Reyes, 1974). Testosterone in umbilical cord blood at birth is also higher in males than females, although this difference appears to be smaller than that seen during the prenatal surge (Herruzo et al., 1993; Keelan et al., 2012). Furthermore, there is a well-documented second surge in testosterone concentrations in boys particularly from about the first to the third month postnatal, with levels then declining to baseline by about 6 months and remaining low until puberty (Bouvattier et al., 2002; Corbier et al., 1992; Forest et al., 1974; Gendrel et al., 1980; Hadziselimović et al., 1986; Hammond et al., 1979; Quigley, 2002; Winter et al., 1976). A recent study (Lamminmäki et al., 2012) also found increased testosterone concentrations in girls during the first weeks of life, followed by a steady decline to baseline by about 6 months postnatal. Although this pattern was similar to that observed in male

infants, testosterone concentrations during early infancy were significantly and consistently lower in girls than in boys.

This early postnatal hormonal surge in human infants, sometimes referred to as “mini-puberty,” occurs during a critical period of neural development, characterized by high cerebral plasticity. The brain, and in particular the cerebral cortex, continues to develop rapidly during the early postnatal period (Gao et al., 2009; Gilmore et al., 2012; Knickmeyer et al., 2008; Li et al., 2015; Petanjek & Kostovic, 2012; Tau & Peterson, 2010). Cortical structure, including cortical thickness and surface area, increases markedly during the first year of life (Li et al., 2015). In addition, boys have been found to show significantly faster rates of surface area expansion compared with girls in 7 cortical regions (Lyall et al., 2015), including the left superior parietal lobule, an area that appears to be activated during mental rotation tasks in adults (Gogos et al., 2010). In addition, the lateral visual/parietal network (V3), a network that appears to be involved during mental rotation tasks in adults (Alivisatos & Petrides, 1997; Jordan et al., 2001; Schendan & Stern, 2007; Schöning et al., 2007), has been found to show rapid development throughout the first six months of life and appears to be adult-like in one-year-old children (Gao et al., 2015). Such periods of rapid growth and development are considered to be highly sensitive to genetic and environmental factors thought to shape human cognitive development (Lyall et al., 2015). These times of increased testosterone exposure (prenatally, and during the early postnatal period of mini-puberty) are therefore the times when testosterone would be hypothesized to influence the development of mental rotation ability.



### **How large are the gender differences in human behavior?**

The magnitudes of human gender differences vary for different behaviors. The largest gender differences are seen in children's play behavior (toy and activity preferences), in sexual orientation (a person's direction of erotic interest) and in gender identity (sense of self as male or female) (Hines, 2015), and differences in these areas appear to be at least as large as the sex difference in height ( $d = 2.0$ ). Perhaps because these gender differences are large, much of the research investigating a possible influence of testosterone on human gender development has focused on these outcomes. Gender differences in cognition are smaller than those in height, and smaller than those in some other behaviors. Nevertheless, the largest of these differences in cognition, that in mental rotation ability, is, as noted before, moderate to large ( $d = 0.56$  to  $0.73$ ), and it is reasonable to hypothesize that testosterone during early critical periods might contribute to this gender difference.

### **How can the influences of testosterone on human gender-related behavior be studied?**

Studies of non-human mammals have used powerful experimental techniques. Animals have been assigned at random to receive hormonal or placebo manipulations. Similar experimental techniques are not ethical in humans. Instead, researchers have looked at the behavior of individuals who were exposed to atypical concentrations of testosterone during early development, for example, because of genetic abnormalities. In addition, researchers have measured testosterone in typically-developing individuals, for example by sampling hormones in amniotic fluid during gestation, in umbilical cord blood at birth, or in blood, saliva, or urine samples during early infancy.

The disorder that has been studied most extensively in regard to human gender development is congenital adrenal hyperplasia (CAH). CAH is a disorder in which the adrenal gland produces increased concentrations of testosterone and other androgens, beginning prenatally. Girls with CAH have elevated androgen concentrations prenatally, whereas androgen concentrations in boys with CAH are similar to those of unaffected boys. Because of their early androgen exposure, girls with CAH might be predicted to show more male-typical behavior. At least a dozen studies have reported increased male-typical childhood play behavior, reduced heterosexual interest, and reduced female gender identity in girls with CAH (Hines, 2015). However, findings regarding mental rotation performance in females with CAH have been inconsistent (Hines, 2015), perhaps because hormones used in the medical treatment of CAH cause reduced working memory and reduced performance on spatial tasks (Browne et al., 2015; Collaer et al, 2016).

Research studying early testosterone exposure in typically developing individuals during early development in relation to later mental rotation performance has been scarce and, like the findings in girls with CAH, results have been inconsistent (Hines, 2009, 2015; Liben et al., 2002). One study found that concentrations of testosterone, measured in umbilical cord blood at birth, negatively predicted spatial ability at the age of 6 years in girls, as measured by a test that included a 2D mental rotation task (Jacklin et al., 1988), a result in the opposite direction than predicted. Another study found that concentrations of testosterone measured in amniotic fluid correlated positively with speed of mental rotation in girls aged 7 years, although not with overall scores (i.e., number correct, which is the outcome measure that has been shown to differ by gender) (Grimshaw et al., 1995). A third study found no relationship between testosterone in amniotic fluid and later mental rotation performance, although amniotic testosterone positively predicted performance on a separate spatial

measure that typically shows a small gender difference (Auyeung, Knickmeyer et al., 2012). Small sample sizes or the use of outcome measures that did not differ by gender could have contributed to these inconsistent outcomes.

As of yet, to our knowledge, no published studies have related testosterone concentrations during mini-puberty to later mental rotation performance, although mini-puberty represents a potential critical period for testosterone to influence neurobehavioral sexual differentiation (Hines et al., 2015).

### **Social and Experiential Influences on Mental Rotation**

Regarding social and experiential influences on mental rotation abilities, infant crawling, childhood play experiences, object manipulation and exploration, spatial experiences, gender stereotypes, and training all appear to contribute. For example, Schwarzer, Freitag, Buckel, & Lofruchte (2013) found that crawling relates to 9-month-old infants' mental rotation abilities, crawling infants being more successful at performing a 3D task than non-crawling infants of the same age.

Gender differences in spatial abilities also have long been thought to be influenced by gender differences in early child play behavior (Baenninger & Newcombe, 1995). Male-typical toys (e.g. vehicles, balls) have been noted to afford opportunities for object manipulation and exploration that are thought to enhance spatial performance (Liss, 1981), whereas female-typical toys (e.g. dolls) are less likely to afford such opportunities (Block, 1983; Campbell et al., 2000). Consistent with this, recent findings suggest that mental rotation abilities in infants correlate with experience of object manipulation and exploration (Möhring & Frick, 2013; Schwarzer, Freitag, & Schum 2013).

In addition, research with preschool children has found positive correlations between play with male-typical toys and spatial abilities (Fagot & Littman, 1976; Serbin & Connor, 1979). Concerning mental rotation in particular, a study with undergraduate students found an association between performance on a 3D mental rotation task and retrospectively recalled experience with spatial toys. However, another study employing a similar method of retrospective assessment (Hines et al., 2003) found no enhanced mental rotation performance in either females or males with CAH, despite both groups recalling high levels of male-typical play in childhood. Negative results for the hypothesized link between male-typical play and mental rotation abilities have also been reported by some other researchers (Caldera et al., 1999; Grimshaw et al., 1995).

In addition to toy preferences, other types of activities and spatial experiences involved in sex-typed play have also been related to mental rotation performance. One study (Voyer & Isaacs, 1993, cited in Grimshaw et al., 1995) reported that participation in sports involving spatial ability positively predicted performance on a 3D mental rotation test in university undergraduates. However, another investigation of the relationship between spatial play and mental rotation performance found no correlation between the two variables in 7-year-old children (Grimshaw et al., 1995).

Mental rotation performance also appears to improve with training (Bruce & Hawes, 2015; Cherney, 2008; Cherney et al., 2003; de Acedo & Ganuza, 2003; Kail & Park, 1990; Kyllonen et al., 1984; Murray, 1995). A meta-analysis examining studies of a broad range of spatial tasks (Baenninger & Newcombe, 1989) found that both men's and women's performances benefitted from spatial activities such as play with manipulative toys or experience with geometry and mathematics, and from medium-duration spatial training. Moreover, even a brief spatial exposure may produce significant changes. For example, one

study found that exposure for 2 minutes to a simple pencil-and-paper rotation task eliminated the gender difference on a more complex 3D mental rotation test (Cherney et al., 2003).

Engagement with computer games also has been found to produce significant improvements in mental rotation performance in both females and males (Cherney, 2008; De Lisi & Wolford, 2002; Feng et al., 2007; Terlecki & Newcombe, 2005).

Masculinity and femininity, as assessed using questionnaires, have also been related to mental rotation performance. For example, high masculinity scores on the Bem Sex-Role Inventory (Bem, 1974) have been found to correlate positively and significantly with performance on mental rotation tasks in both males (Jagieka & Herman-Jeglinska, 1998) and females (Scarborough & Johnston, 2005). Other studies have also found correlations between masculine personality traits and better performance on mental rotation tasks, and a meta-analysis of 12 such studies (Reilly & Neumann, 2013) found a positive and significant association between masculine personality traits and mental rotation abilities.

In summary, although we do not yet have a complete understanding of the causes of the gender difference in mental rotation performance, a link with gonadal hormones, particularly testosterone during early development, can reasonably be hypothesized. In addition, a broad range of social and experiential factors has been found to relate to mental rotation performance in children and adults.

### **Present Study**

The present study investigated the relationship of two types of factors, early postnatal testosterone exposure during mini-puberty and parental attitudes regarding gender, to mental rotation performance in 5 – 6 month-old infants. We measured testosterone during the early postnatal period because: (i) testosterone is higher in boys than in girls at this time,

suggesting that it is an important period for neurobehavioral gender development; (ii) brain regions that are thought to relate to mental rotation are developing at this time, and (iii) biological samples can be obtained relatively unintrusively at this time. We measured parental influences because these are the types of social influences that are likely to be most important during infancy. We tested two hypotheses in typically-developing girls and boys: (1) early postnatal testosterone exposure predicts mental rotation performance; and (2) parental attitudes regarding gender predict mental rotation performance.

### **Methods**

To test these hypotheses, we measured salivary testosterone and mental rotation performance in healthy, full term infants. We also assessed attitudes regarding gender in their parents. Saliva samples were obtained to measure testosterone when infants were 1 – 2.5 months of age, and mental rotation performance was assessed at 5 – 6 months of age. At this second time-point, a second sample of saliva also was collected and parents' gender-related attitudes were assessed using the Child Gender Socialization (CGS) Scale (Blakemore & Hill, 2008).

### **Participants**

Participants were 61 healthy infants (29 males, 32 females) and 59 mothers and 3 fathers (for one of the infants, both parents took part), recruited from the Cambridgeshire, UK area. The infants' ages ranged from 29 to 71 days ( $M = 50.57$ ,  $SD = 7.81$ ) at the first visit, and from 145 to 191 days ( $M = 166.44$ ,  $SD = 9.90$ ) at the second visit. None of the parents reported any visual or hearing problems in any of the infants.

The mothers' ages, at the time of their child's birth, ranged from 24.49 to 42.85 years ( $M = 33.44$ ,  $SD = 4.01$ ). Regarding ethnicity, 96.7% of the women were Caucasian, 1.6%

were Indian, Pakistani or Bangladeshi, and 1.6% were of mixed background. Regarding education, 70.5% of the mothers had a postgraduate degree, 27.9% had a university degree or vocational training, and 1.6% had no post-secondary education.

Information about all fathers (including those who did not take part in the study directly) was also collected. The fathers' ages, at the time of their child's birth, ranged from 25.10 to 49.56 years, ( $M = 35.25$ ,  $SD = 4.97$ ). Regarding ethnicity, 90.2% of the fathers were Caucasian, 1.6% were Indian, Pakistani or Bangladeshi, 4.9% were of mixed background, and 3.3% were of other ethnicity. Regarding education, 63.9% of the fathers had a postgraduate degree, 34.4% had a university degree or vocational training, and 1.6% had no post-secondary education.

## **Procedures**

Data concerning salivary testosterone were collected for 60 of the initial 61 infants at time point one (one infant was excluded due to insufficient saliva), and for 58 of the 61 infants at time point two (three infants were excluded due to insufficient saliva). Data concerning mental rotation were obtained for 54 (26 males, 28 females) of the 61 infants tested (seven infants were excluded because they refused to watch the video). Data for both testosterone concentrations and mental rotation were obtained for 53 (25 males, 28 females) of the 61 infants tested, and data for both the mental rotation task and the CGS Scale were obtained for 54 (26 males, 28 females) of the 61 infants tested.

### **Saliva collection.**

Saliva was collected using the Salimetrics Infant's Swab (SIS), a commercially available salivary collection tool developed by Salimetrics LLC, State College, PA. The SIS is an inert polymer swab whose small diameter makes it suitable for use with infants younger

than 6 months of age. The length of the swab allows one end to be held by the researcher while the other end is in the infant's mouth, eliminating any choking hazard. The swab has no smell or taste, and its texture is acceptable to children. The swab has very good recovery rates (typically in the range of 200-1000 $\mu$ L), and has been shown to be free from interference. The kit's sensitivity is reported at 1 pg/ml, and the assay range is reported as 6.1–600 pg/ml (Salimetrics LLC, 2016). The average inter- and intra-assay precision coefficients of variation are low with no deleterious matrix effects, and salivary testosterone concentrations have been found to correlate highly with serum testosterone concentrations,  $r(26) = 0.96$ ,  $p < 0.001$  (Salimetrics LLC, 2016).

Saliva collection was performed by the same trained researcher for all the infants, in accordance with a standardized protocol (Salimetrics LLC, 2015). Saliva samples were always collected early in the day, typically between 10 a.m. and 12 noon (and never later than 12 noon), because testosterone exhibits a diurnal rhythm, with the highest concentrations in the morning and the lowest concentrations around midnight (Ankarberg & Norjavaara, 1999; Diver et al., 2003). We also collected saliva samples at least one hour after the infant's last feeding, to eliminate the risk of contamination by hormones present in milk or in baby formula (Granger et al., 2007; Magnano et al., 1989; Salimetrics LLC, 2015). After collection, samples were stored at -80°C until analyzed. Upon thawing, salivary samples were vortexed and then centrifuged to separate the saliva from solid particles. Testosterone concentrations were measured using radioimmunoassay by Salimetrics Europe Ltd., Biomarker Analysis Laboratory, Cambridge, U.K. Intra-assay variability was less than 10%.



**Mental rotation task.**

Mental rotation ability was assessed using the procedure developed by Moore and Johnson (2008). The task design uses a looking-preference paradigm, and relies on the well-established observation that infants typically spend more time looking at novel than familiar stimuli, following a period of habituation.

***Apparatus and setting.***

Infants were tested individually while sitting on a parent's lap in a darkened room. A 53 cm monitor screen displayed the stimuli at a distance of 100 cm. The stimuli presentations were initiated and controlled by an observer located in a different room using a computer keyboard. The observer was blind to the specific stimulus being shown, but could see the infant without being seen via a two-way mirror. To avoid potential influences on the infants' behavior, parents were asked to keep their eyes closed during the procedure. Data regarding mental rotation performance were collected using the Habit software (Cohen, Atkinson, & Chaput, 2002). The software presented stimuli, timed trials, calculated the habituation criterion, and stored data. All sessions were recorded on compact disc using a digital camcorder. To establish inter-rater reliability, 15% of all sessions were randomly selected and coded off-line by a second independent observer. To measure reliability, the interclass correlation coefficient (ICC) was employed (Kottner et al., 2011). The agreement between the two observers was strong (ICC = .998).

***Stimuli.***

The stimuli were video representations of dynamic 3D objects, depicted in rotational movement around their vertical axis in 3D space. They consisted of simplified Shepard-Metzler objects and their mirror images, presented on a black background (see Fig. 1).

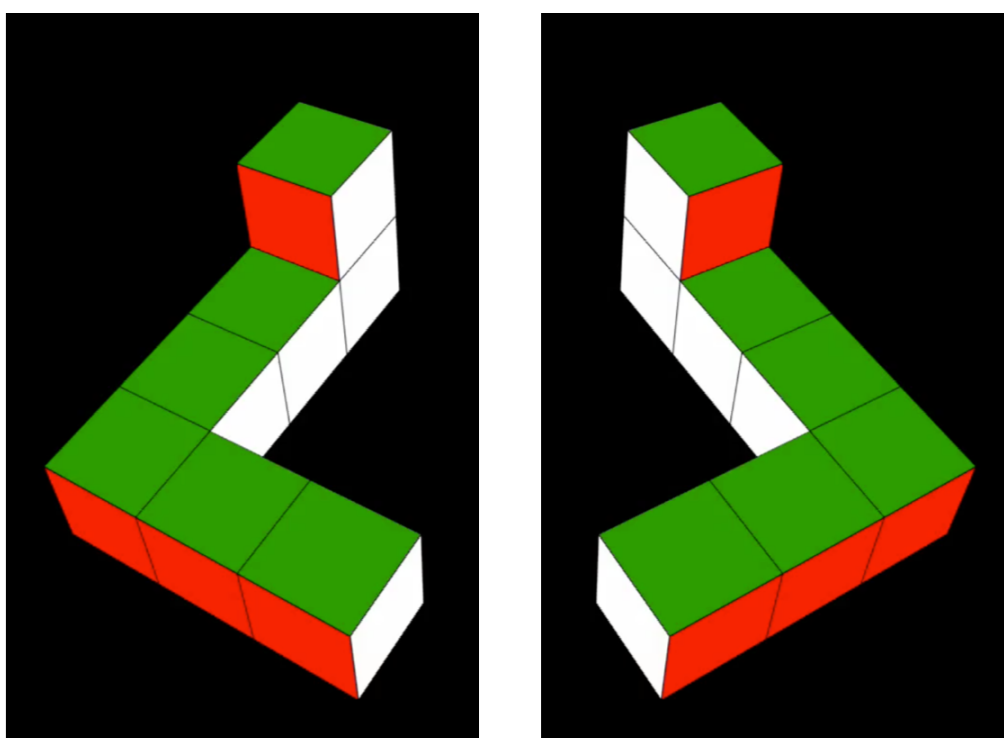


Fig. 1. Simplified Shepard-Metzler objects (from Moore & Johnson, 2008). The object shown on the left is arbitrarily called the L-object, and its mirror image, shown on the right, is arbitrarily called the R-object.

More specifically, these were abstract objects made up of seven cubes, with each cube joined by a common facet with another, forming 90° bends at the top and bottom as follows: a two-cube bar (x-axis), attached at the bottom of a straight central bar consisting of four cubes (y-axis), followed by a single-cube bar (z-axis) attached to the top of the central bar. Seen from above, all visible faces of the objects were yellow; seen from below, all visible faces were red. Seen from the front, right, back, and left, the faces were purple, blue, white, and green, respectively. As in Moore and Johnson (2008), the object shown on the left hand side in Fig. 1 was (arbitrarily) called the L-object, and the mirror image shown on the right was called the R-object.

### ***Procedure.***

The test trials were preceded by habituation trials, during which the infants were presented with videos showing either the L-object or the R-object revolving back and forth through a nearly 240° arc, at 45° per second. The rotations were looped, so that upon reaching its maximum extent of rotation, the object would rotate back toward its starting point. During the habituation stage, each infant was randomly assigned to either the L- or the R-habituation group and saw a series of identical videos showing L- or R-objects only, until habituation occurred. Habituation was evaluated by computing the average fixation duration for the first four habituation trials and then comparing this to the average fixation duration across subsequent four-trial blocks. When the average fixation duration in a given four-trial block declined to 50% or less of that computed for the first four trials, the infant was deemed to have habituated to the stimuli. Test trials were administered after infants habituated, or after 12 habituation trials, whichever came first. In the test trials, novel videos of the L- and R-objects' "back sides" were presented to each infant, showing L- or R-objects continuing the

rotation presented in habituation videos through a previously unseen nearly 120° of arc, starting 1.5° apart from the maximum rotation angle shown in the habituation video (see Fig. 2). As in the habituation videos, the rotations in the test videos were looped so that the objects moved back and forth. Six test trials were presented to each infant. Half of the participants were randomly assigned to see the L-object in the first test trial; the other half saw the R-object first. The stimuli then alternated in subsequent test trials between R- and L-objects, always shown from the novel perspective. All trials were preceded by the presentation of an attention-getter, designed to draw the infant's attention to the screen. The trials were initiated with a computer key stroke by the observer. They ended 2 seconds after a subsequent key stroke by the observer indicated that the infant was no longer fixating on the stimulus, or after 60 seconds, whichever came first. The 2-second interval was meant to allow the infant to return attention to the stimulus, in which case the trial would continue. Otherwise, the attention-getter was employed again and a new trial ensued.

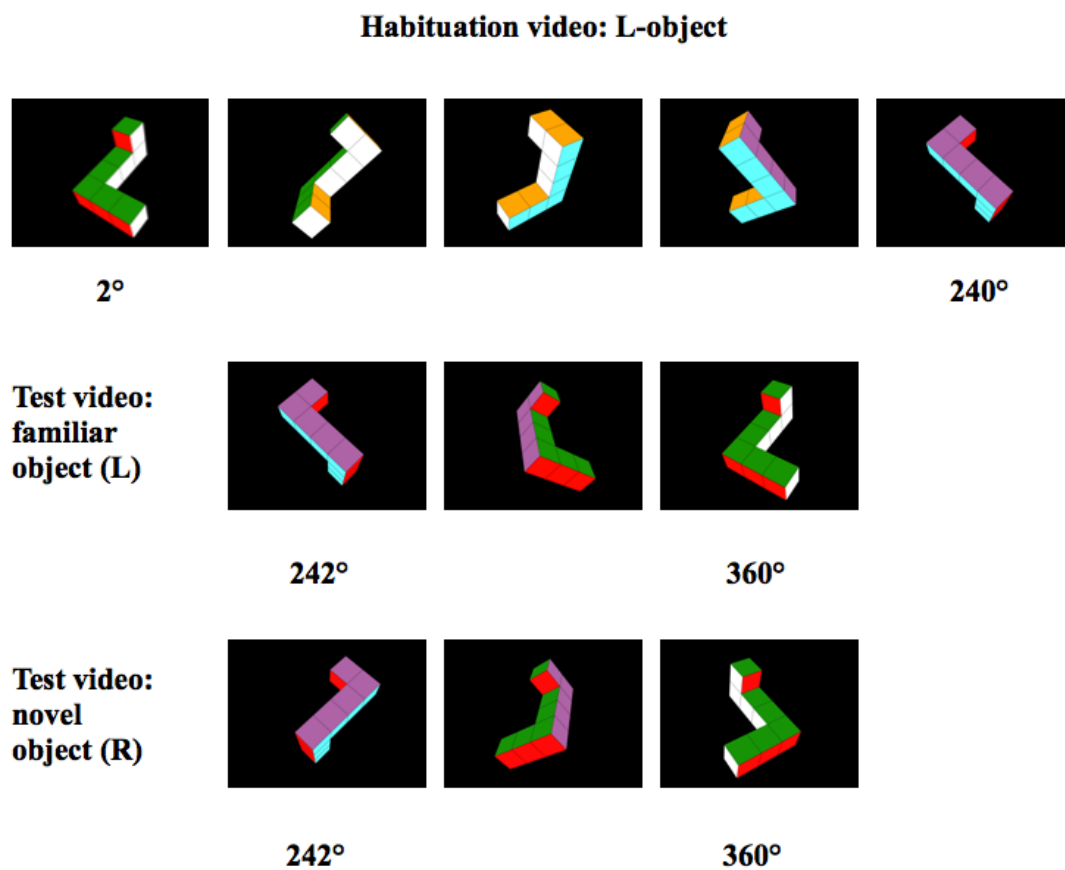


Fig. 2. Still frames showing different rotation angles of the stimuli, as presented in the habituation and test videos. In the habituation video, the L-object moved back and forth through a nearly 240° arc. The test video then presented either the familiar L-object or its mirror image (the R-object), rotating back and forth through a previously unseen nearly 120° arc.

### **The 'Child Gender Socialization Scale'.**

The CGS Scale (Blakemore & Hill, 2008) was used to assess levels of gender-stereotypical parental attitudes, by asking parents questions about various activities in which their children might engage either at present or later in life (e.g., playing with toy cars, taking ballet lessons). The measure consists of 28 items, arranged on five subscales (Toys and Activities Stereotyped for Girls, Toys and Activities Stereotyped for Boys, Helping at Home, Education for Marriage and Family, and Disapproval of Other-Gender Characteristics), which differentiate between the parents of boys and the parents of girls, as well as between traditional and less-traditional parents. Responses are made on a Likert 7-point rating scale ranging from "very negative" to "very positive." The first subscale is typically scored higher by parents of girls, and the rest of the subscales are typically scored higher by parents of boys. The subscales are reported to show internal consistencies of  $\alpha = .60$  to  $\alpha = .93$ , and test-retest reliabilities of  $\alpha = .65$  to  $\alpha = .76$  (Blakemore & Hill, 2008). In addition, child gender is a significant predictor of scores on this measure, whereas the gender of the parent completing the measure is not (Blakemore & Hill, 2008).

### **Ethical Approval**

This study received ethical approval from the Cambridge University Human Biology Committee. In return for their participation, parents received a small gift for the infant.

## **Results**

### **Testosterone Concentrations**

As shown in Fig. 3, testosterone concentrations were significantly higher in boys than in girls at age 1 – 2.5 months postnatal ( $M = 79.53$  pg/ml,  $SD = 23.51$  for males;  $M = 67.82$  pg/ml,  $SD = 18.97$  for females;  $t(58) = 2.13$ ,  $p = .04$ , Cohen's  $d = 0.54$ ). Given the variation

in the infants' ages at the first visit, a one-way ANCOVA was conducted to explore the effect of sex on testosterone concentrations whilst controlling for age. Levene's test and normality checks were carried out and the assumptions met. There was a significant sex difference in testosterone concentrations,  $F(1,58) = 7.66, p = .008$ .

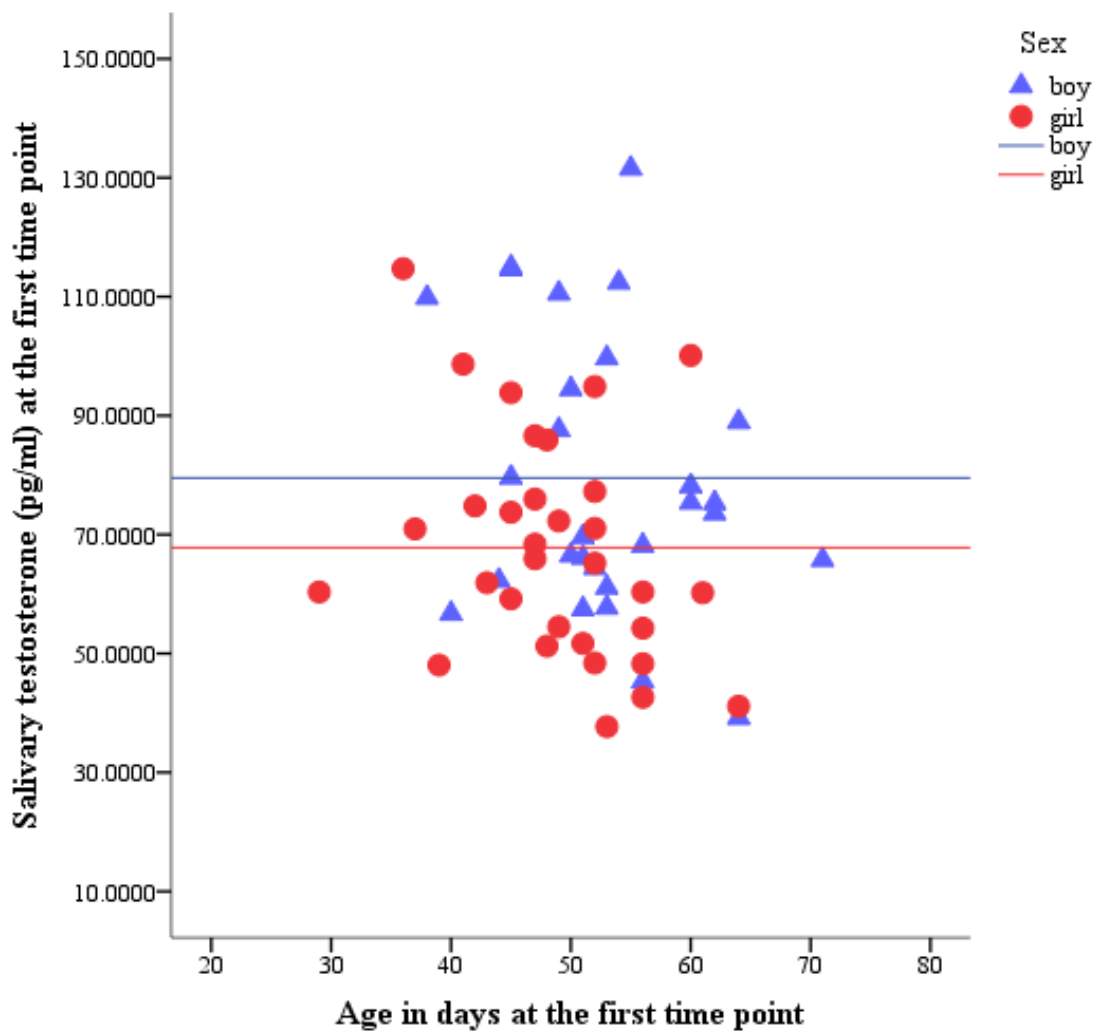


Fig. 3. Salivary testosterone (pg/ml) at 1 – 2.5 months (individual and mean values).



In contrast, at age 5 – 6 months, testosterone concentrations were significantly lower in both sexes than they were at the first visit [ $t(25) = 6.89, p < .001, d = 1.63$ , for males;  $t(30) = 5.46, p < .001, d = 1.21$ , for females], and, as shown in Fig. 4, there was no significant sex difference in testosterone concentrations at age 5 – 6 months ( $M = 47.46$  pg/ml,  $SD = 14.83$  for males;  $M = 47.26$  pg/ml,  $SD = 14.49$  for females;  $t(56) = .05, p = .96, d = 0.01$ ).

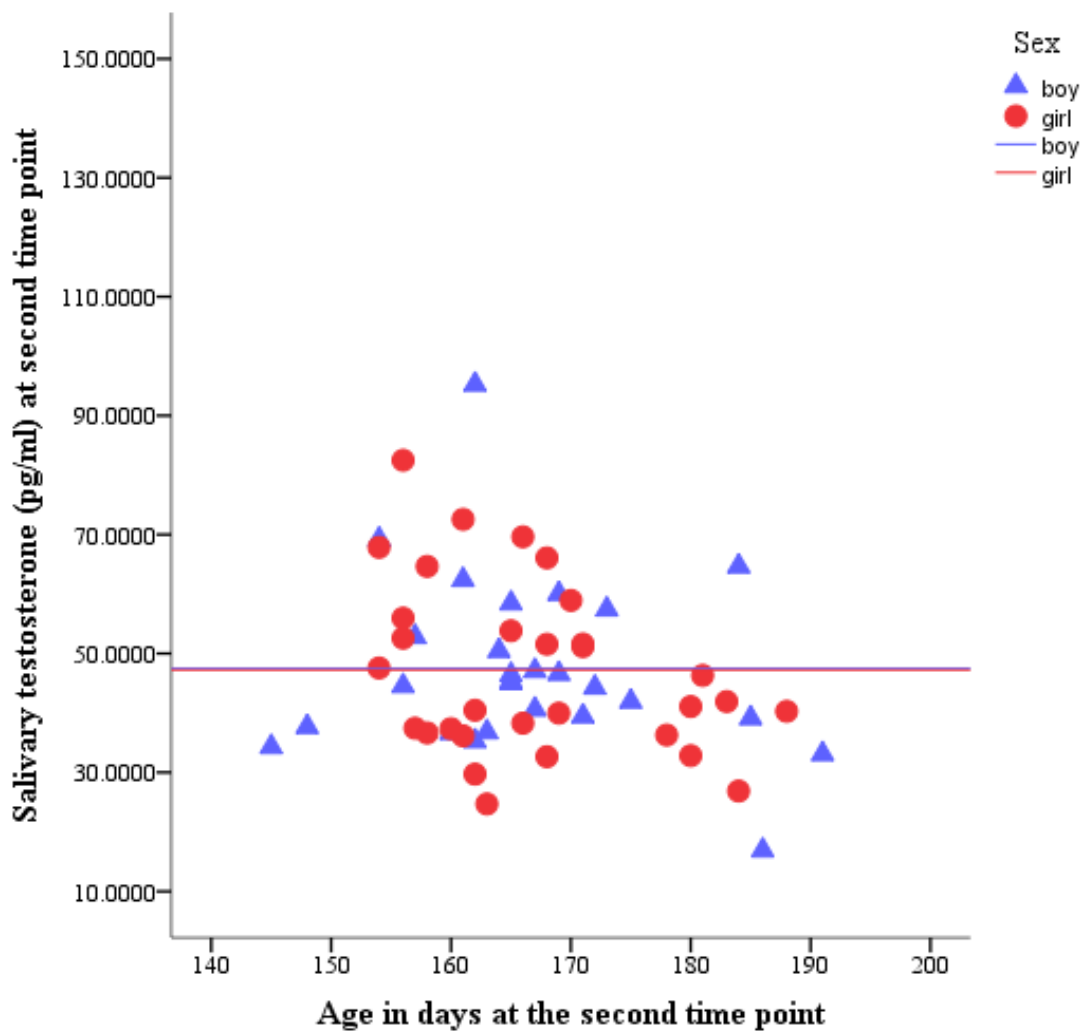


Fig. 4. Salivary testosterone (pg/ml) at 5 – 6 months (individual and mean values).

### **Mental Rotation Measures**

The principal dependent measure of mental rotation performance was a post-habituation novelty preference for the L- or R-object. As shown in Fig. 5, male infants spent a significantly longer time looking at the novel stimulus than they did looking at the familiar one,  $t(25) = 3.37, p = .002, d = 0.85$ , and 65% of the male infants preferred the novel stimulus. In contrast, female infants looked at the familiar and novel test stimuli about equally,  $t(27) = -.10, p = .92, d = 0.2$ , and 46% of the female infants preferred the novel stimulus. These findings suggest that more male than female infants had developed an ability for mental rotation at 5 – 6 months of age.

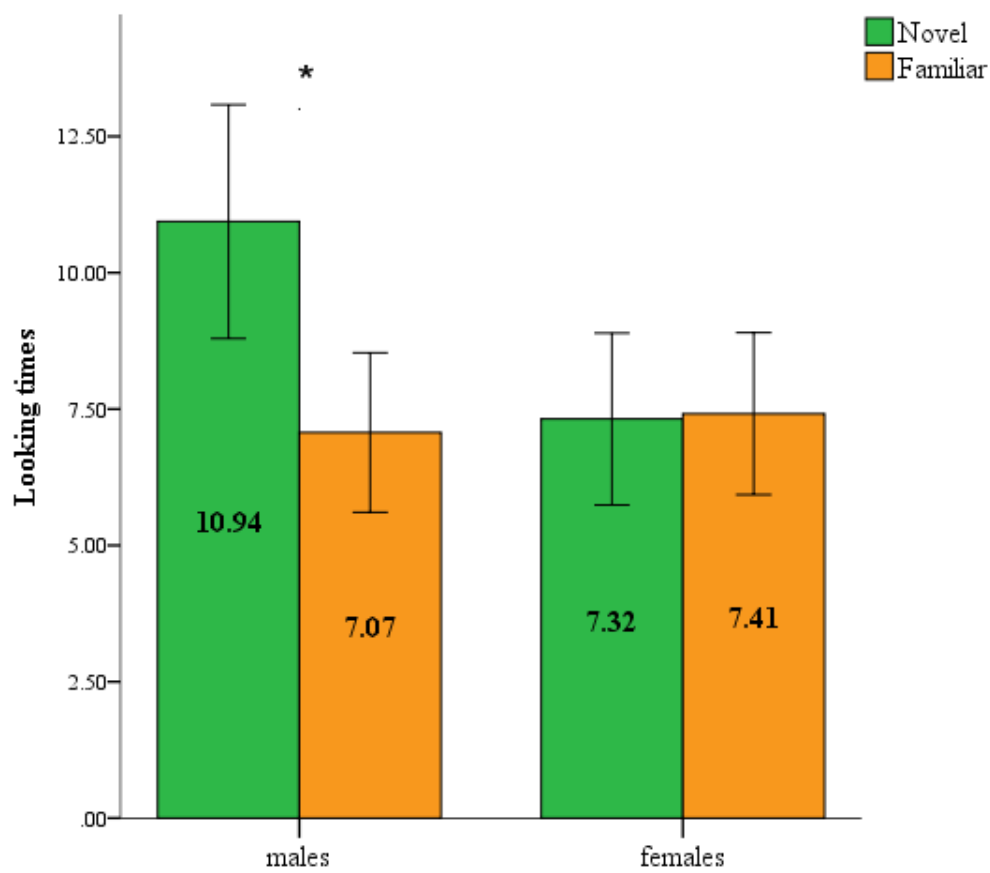


Fig. 5. Five to six-months-old infants' looking times (in s) at the novel and familiar stimuli

To obtain a quantitative measure of mental rotation ability for each infant, we calculated novelty preference scores. The novelty preference score was the ratio of time spent looking at the novel stimulus to time spent looking at both test stimuli. The results were then converted to percentage scores as in other studies (Moore & Johnson, 2008, 2011).

For novelty preference, we found a significant gender difference. The male infants' novelty preference was significantly greater than that of the female infants ( $M = 59.47$ ,  $SD = 15.66$  for males;  $M = 49.16$ ,  $SD = 16.30$  for females),  $t(52) = 2.36$ ,  $p = .02$ ,  $d = 0.64$ . Given the variation in the infants' ages at the second visit, a one-way ANCOVA was conducted to explore the effect of gender on novelty preference whilst controlling for age. Levene's test and normality checks were carried out and the assumptions met. There was a significant gender difference in mental rotation,  $F(1,52) = 5.50$ ,  $p = .02$ .

### **Links between Testosterone Concentrations and Infants' Mental Rotation Abilities**

Salivary testosterone concentrations at 1 – 2.5 months of age correlated significantly with novelty preference scores on the 3D mental rotation task at age 5 – 6 months when data for girls and boys were analyzed together ( $r = .28$ ,  $p = .03$ ). When data were analyzed within each sex separately, testosterone concentrations at 1 – 2.5 months of age correlated significantly with novelty preference scores on the 3D mental rotation task in 5- to 6-month-old boys ( $r = .49$ ,  $p = .01$ ), but not in 5- to 6-month-old girls ( $r = -.06$ ,  $p = .78$ ) (as shown in Fig. 6 and in Fig. 7).

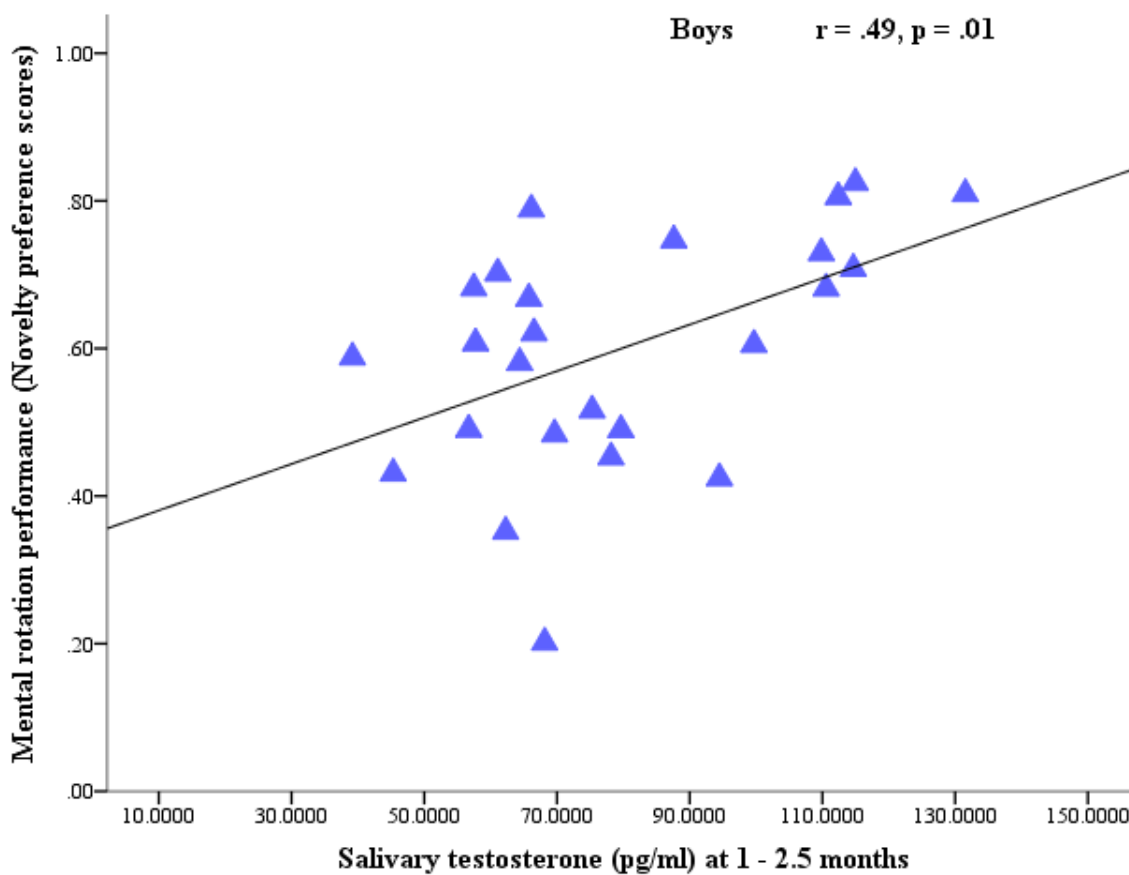


Fig. 6. Testosterone concentrations (age 1 – 2.5 months) and mental rotation performance (age 5 – 6 months) in boys

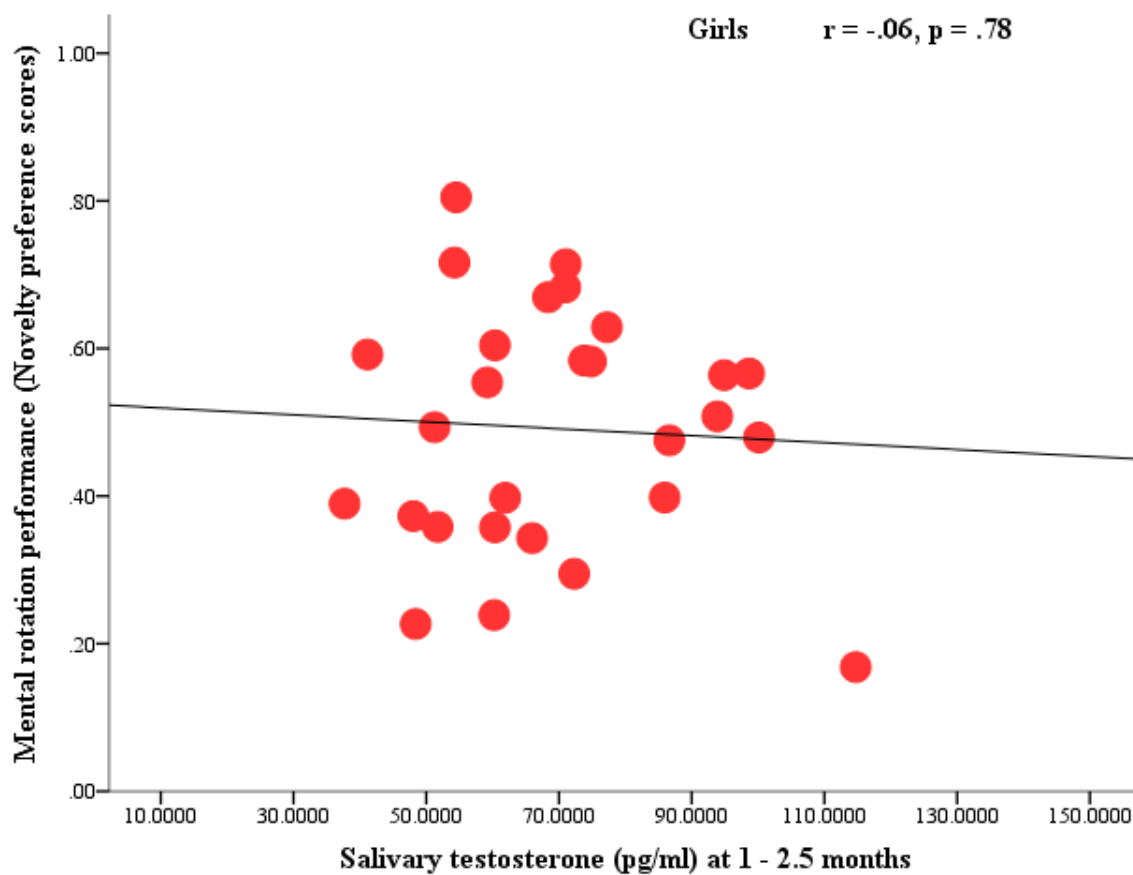


Fig. 7. Testosterone concentrations (age 1 – 2.5 months) and mental rotation performance (age 5 – 6 months) in girls

### **Parental Attitudes concerning Gender Roles**

The internal reliabilities of the 5 scales that differentiated between boys' parents and girls' parents ranged from  $\alpha = .73$  to  $\alpha = .89$ . As expected, the questionnaire reliably distinguished between boys' parents and girls' parents, consistent with results reported by Blakemore and Hill (2008).

Boys' parents scored significantly higher than girls' parents on the following subscales: Toys and Activities Stereotyped for Boys  $t(55) = 2.47, p = .016, d = 0.67$ ; Helping at Home  $t(55) = 3.10, p = .003, d = 0.81$ ; Education for Marriage and Family  $t(55) = 6.10, p < .001, d = 1.63$ . Boys' parents also scored higher than girls' parents on the Disapproval of Other-Gender Characteristics subscale, but the difference did not reach conventional statistical significance,  $t(55) = 1.43, p = .16, d = 0.38$ . For the Toys and Activities Stereotyped for Girls subscale, girls' parents scored slightly but non-significantly higher than boys' parents,  $t(55) = -.24, p = .81, d = 0.06$ .

### **Links between Parental Attitudes concerning Gender and Infants' Mental Rotation**

#### **Abilities**

We assessed the relationship of parental attitudes to infants' mental rotation abilities by conducting separate analyses for boys and for girls, using each of the 5 subscales of the CGS Scale, yielding a total of 10 correlational analyses.

Pearson analyses showed a significant negative correlation between scores on the Disapproval of Other-Gender Characteristics subscale and the novelty preference scores for girls ( $r = -.57, p = .002$ ), but not for boys ( $r = .15, p = .46$ ) (as shown in Fig. 8 and in Fig. 9). The direction of this correlation suggests that the more traditional parents reported being (e.g. by disapproving of their daughters playing with boys' toys or acting like boys), the worse



their daughters performed on the mental rotation task. Spearman correlations confirmed these findings, showing a significant correlation between scores on the Disapproval of Other-Gender Characteristics subscale and novelty preference scores in girls ( $\rho = -.54, p = .003$ ), but not in boys ( $\rho = .10, p = .62$ ).

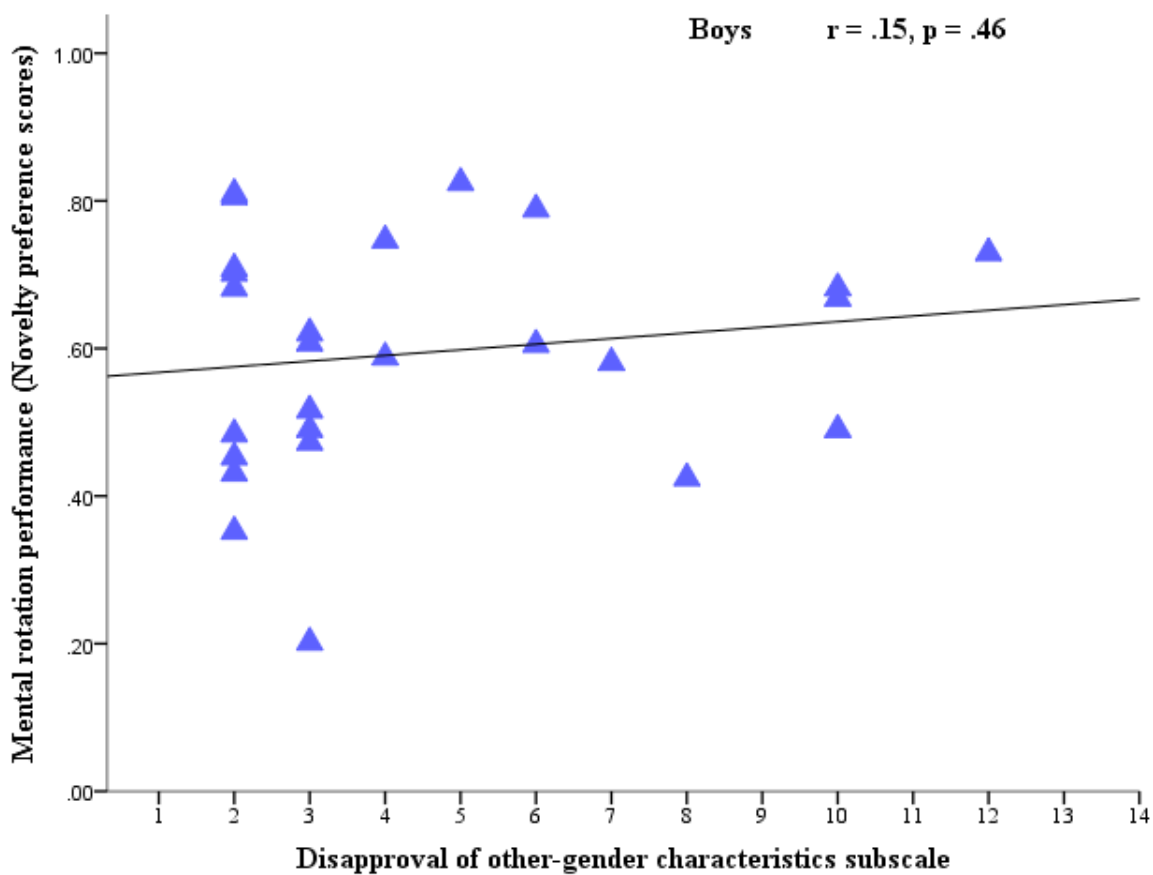


Fig. 8. Disapproval of other-gender characteristics subscale and mental rotation performance in boys

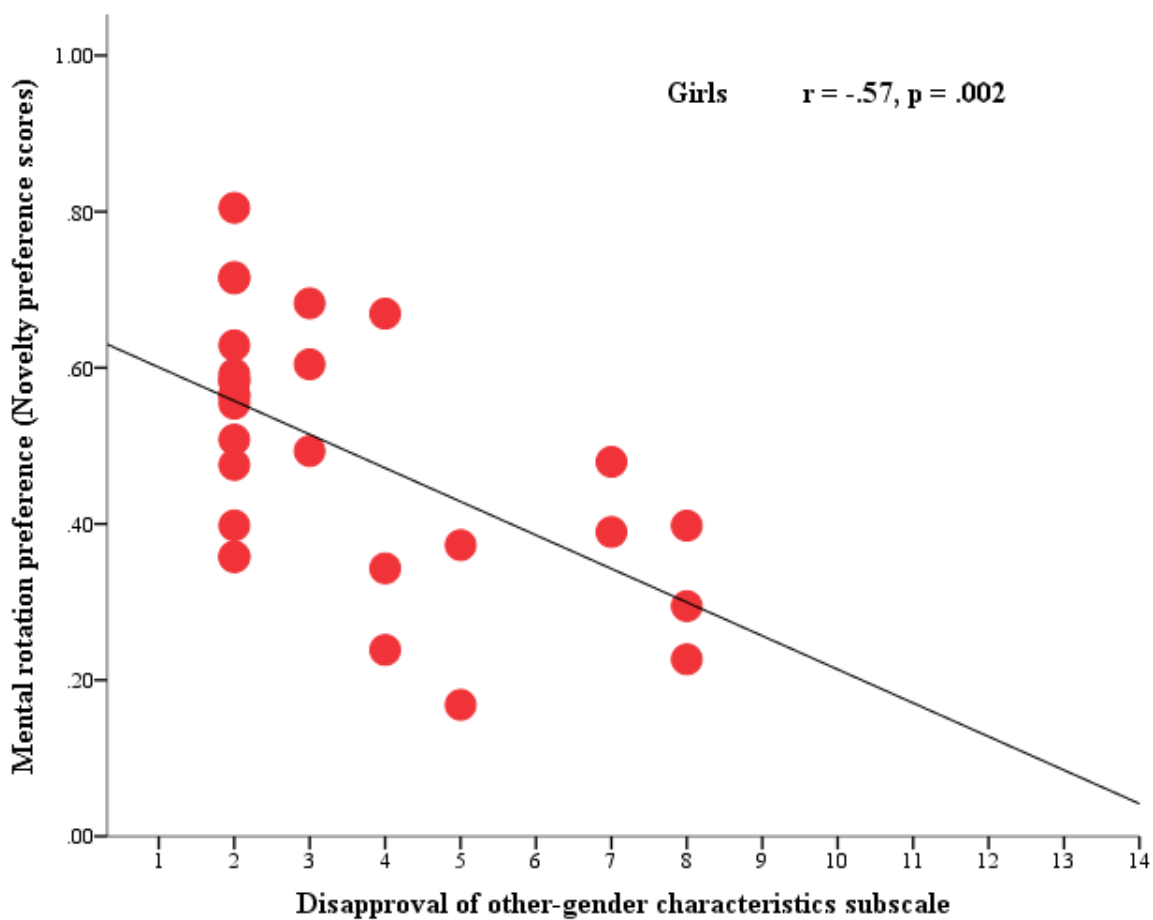


Fig. 9. Disapproval of other-gender characteristics subscale and mental rotation performance in girls

No correlations between the other subscales and mental rotation performance were significant for either girls or boys (see Table 1).

Table 1: Correlations between mental rotation scores and Child Gender Socialization

Mental rotation scores/Child Gender Socialization Scale	Boys			Girls		
	<i>N</i>	<i>r</i>	<i>P</i>	<i>n</i>	<i>r</i>	<i>p</i>
Toys and activities stereotyped for girls	26	.029	.886	28	-.013	.948
Toys and activities stereotyped for boys	25	-.290	.159	28	.002	.991
Helping at home	26	.032	.877	28	.153	.436
Education for marriage and family	26	.230	.258	28	-.210	.283
Disapproval of other-gender characteristics	26	.152	.460	28	-.567**	.002

n Scale scores in boys and girls\*\* Correlation is significant at the 0.01 level (2-tailed)

## Discussion

This study makes several novel contributions to understanding the factors influencing mental rotation performance in early infancy. First, it is the first study to provide evidence of a correlation between testosterone concentrations during the early postnatal testosterone surge (mini-puberty) and later performance on a mental rotation task in boys. Second, it is the first study to provide evidence of possible influences of parental attitudes on infants' mental rotation performance in girls. Third, it is also the first study to provide evidence of an early postnatal hormone surge using assays of testosterone in saliva, rather than blood or urine samples.

### Gender Differences in Infants' Mental Rotation Abilities

The study found a moderate-to-large gender difference in infants' mental rotation abilities at 5 – 6 months, with male infants more likely to show mental rotation ability than female infants. This finding is consistent with findings from several other studies of mental rotation in infancy (Moore & Johnson, 2008, 2011; Quinn & Liben, 2008, 2014). Of these, Moore & Johnson (2008) is particularly relevant, as it is the only other study to have reported gender differences in 3D mental rotation in infants of a similar age. The effect size of the gender difference we observed ( $d = 0.64$ ) is similar to that reported by Moore and Johnson ( $d = 0.66$ ). In addition, analyzing within each sex, we found that male infants looked significantly more at the novel stimuli than they did at the familiar stimuli, again suggesting that they had developed 3D mental rotation abilities. This result also is consistent with the prior findings of Moore and Johnson (2008) using the same procedures, and the effect size of the within-sex difference in our study for boys ( $d = 0.85$ ) was slightly larger than that reported by Moore and Johnson ( $d = 0.61$ ). Also in line with Moore and Johnson (2008), we

found that girls did not prefer to look at the novel stimuli over the familiar stimuli (or fewer girls than boys did so), suggesting that, taken as a group, they did not distinguish between the stimuli and therefore did not yet possess 3D mental rotation abilities. Our data also suggest a slight, but non-significant, tendency for girls to look longer at the familiar stimuli than at the novel stimuli, which again resembles the findings of Moore and Johnson (2008).

Not all previous studies of mental rotation abilities in infancy reported gender differences, however. For instance, two studies (Hespos & Rochat, 1997; Rochat & Hespos, 1996) found that infants showed mental rotation abilities as early as 4 months of age, but the researchers did not provide analyses of gender differences in performance. If, however, both boys and girls engaged in mental rotation in these studies, this might have occurred because the task employed was less difficult than ours in two respects: first, it involved a 2D representation of a simpler, T-shaped object; second, the novel test stimulus consisted of an inversion rather than a mirror image of the habituation object. In contrast, when infants of similar ages were exposed to a more difficult variant of this 2D task (in which the novel test stimulus was a mirror image of the habituation object), gender differences were observed (Quinn & Liben, 2008, 2014).

In another study, Möhring and Frick (2013) found that neither boys nor girls were able to perform mental rotation on a 3D task at 6 months of age, unless they were given an opportunity for manual exploration of the stimuli before the test. There are two potential explanations for the apparent inconsistency here. First, the boys and girls who did succeed at mental rotation were given equal opportunities during the study for manual exploration, thus eliminating one potential source of gender differences. Second, the task employed by Möhring and Frick was more difficult than ours, as it required the infants to initiate mental

rotation on their own, instead of allowing them to track an actual rotational event before the test.

Similar points apply to two other studies that found no gender difference in infants' mental rotation abilities. Schwarzer and colleagues investigated the effect of crawling (Schwarzer, Freitag, Buckel, & Lofruthe, 2013) and manual object exploration (Schwarzer, Freitag, & Schum, 2013) on 3D mental rotation in a sample of 9-month-olds, using simplified Shepard-Metzler objects. These researchers found no sex differences in mental rotation abilities; neither boys nor girls were able to perform mental rotation unless they were already crawling or were given the opportunity to manually explore 3D objects before the test. Again, the task used in these studies differed from ours in at least two respects. First, it involved a rotation around the horizontal rather than the vertical axis. Second, the color-patterns on the objects' surfaces varied in ways that may have made it more difficult for the infants to discriminate between different objects.

Summarizing, we suggest that when other studies have not observed gender differences in infants' mental rotation abilities consistent with those reported here, this may have been due to one of two factors: either the mental rotation tasks employed were too simple, in which case boys and girls alike were able to perform them successfully; or the tasks were too complex, in which case neither boys nor girls were able to perform them unless they were given prior opportunities for object exploration and manipulation. Furthermore, another significant factor is that in these latter studies, boys and girls were given equal opportunities for object exploration and manipulation. This may be different from real life, where social factors like differential child play and parental attitudes could be influential.



### **Testosterone and Mental Rotation Abilities in Infancy**

The present study provides evidence of a correlation in boys between concentrations of salivary testosterone at 1 – 2.5 months of age, during mini-puberty, and later mental rotation abilities at 5 – 6 months of age. The existence of such a correlation has been hypothesized before (Moore & Johnson, 2008), but not directly investigated. This result suggests that the early postnatal surge of testosterone (mini-puberty) may have organizational influences on brain development that contribute to later mental rotation abilities in boys. In addition to measuring testosterone at the height of the early postnatal surge, we measured testosterone on the same day that we measured mental rotation. This procedure allowed us to distinguish organizational influences of testosterone, which are long lasting, from possible activational influences, which are transient. Our observation of no relationship between testosterone concentrations at age 5 – 6 months and concurrent mental rotation performances suggests that the relationship between testosterone concentrations at 1 – 2.5 months, at the height of mini-puberty, and mental rotation performances several months later reflects an organizational effect, rather than a more temporary, activational effect.

Although testosterone at age 1 – 2.5 months related to later mental rotation performance in boys, no significant correlation between salivary testosterone and mental rotation abilities was seen in girls. This may have occurred because testosterone is lower during mini-puberty in girls than in boys. The Salimetrics testosterone assay also appears to perform better for boys than for girls, as evidenced by a higher correlation with serum testosterone in boys ( $r = .91$ ) than in girls ( $r = .61$ ) (Salimetrics LLC, 2016).

### **Parental Attitudes concerning Gender and Mental Rotation Abilities in Infancy**

This study also found evidence of a correlation between girls' 3D mental rotation abilities and parental attitudes toward gender roles. We found a significant negative correlation between girls' parents' responses to questions on the Disapproval of Other-Gender Characteristics subscale and girls' performance on the 3D mental rotation task at 5 – 6 months of age. The more traditional the parents' attitudes toward their daughters' playing with boys' toys and acting like boys, the worse the daughters performed on the 3D mental rotation task. We obtained measures for 5 subscales assessing parents' gender-related attitudes, but only one of these subscales related to mental rotation performance. Therefore, this single finding could be spurious. On the other hand, the finding is in line with the results of a recent study which found that mothers' scores on the same subscale correlated negatively with their daughters' rejection of traditional gender roles and with career aspirations at age 18 – 24 years (Colaner & Rittenour, 2015). If our finding is reliable, it might suggest that parents with strongly traditional attitudes to gender treat their daughters in ways that delay or impair the development of their mental rotation abilities.

It should be noted that the questionnaire was completed in most cases (58 out of 61) by the infants' mothers, which reflects the fact that an infant's primary caretaker in the first months of life is still usually the infant's mother. Nevertheless, although child gender is a significant predictor of scores on this measure, parent gender is not (Blakemore & Hill, 2008). Thus, we would not expect different results for the questionnaire if it had been completed by the father.

### **Sex Differences in Salivary Testosterone Concentrations during Mini-Puberty**

Finally, we observed a moderate-to-large sex difference in salivary testosterone concentrations at 1 – 2.5 months of age, with testosterone concentrations significantly higher in boys than in girls. Other research groups also have reported salivary testosterone concentrations during infancy (Alexander et al., 2009; Alexander & Saenz, 2011; Auyeung, Ahluwalia et al., 2012; Caramaschi et al., 2012). None of these studies found a sex difference in testosterone concentrations, however. All of these prior studies collected salivary testosterone from infants older than our participants (3 months and older in Auyeung, Ahluwalia et al., 2012; approximately 4 months in Alexander et al., 2009 and Alexander & Saenz, 2011; and 5.5 months in Caramaschi et al., 2012). We found that testosterone concentrations declined between our first time point (age 1 to 2.5 months) and our second time point (age 5 to 6 months) in both boys and girls, and that there was no longer a sex difference at the second time point. Thus, the prior studies that measured infants' salivary testosterone might have collected their samples when children were too old and the sex difference in testosterone was no longer measurable. There is therefore no direct inconsistency between these findings and ours: indeed, we also found no significant sex difference in concentrations of testosterone at 5 – 6 months of age.

Our finding of a sex difference in testosterone concentrations at the first time point (1 – 2.5 months), but not the second (5 – 6 months), is also consistent with what is known about testosterone concentrations in serum and urine samples in the first six months after birth. Like researchers investigating testosterone concentrations in blood or urine, we found that testosterone concentrations were lower at age 5 – 6 months than they were at age 1 – 2.5 months, both in males and in females. Testosterone concentrations in plasma and urine appear to peak in human infants between about months 1 – 3 postnatally and then decline to baseline

by 6 months of age. This early postnatal testosterone surge has been seen in previous research using serum (Bouvattier et al., 2002; Corbier et al., 1992; Forest et al., 1973; Forest et al., 1974; Pang et al., 1979) and urine (Lamminmäki et al., 2012) samples, and our data suggest that it also can be detected using saliva samples in infants aged 1 – 2.5 months.

### **Limitations and Future Directions**

This is the first study to report correlations between infants' mental rotation abilities and hormonal influences or parental attitudes to gender. However, it is well known that conclusions about causal relations cannot be drawn based on correlational data (Curran & Bauer, 2011). Future research could investigate whether these correlations reflect causation or are mediated by other factors.

Also, at this stage, additional research would be needed to determine whether the correlation we found between early postnatal testosterone and later mental rotation performance in infancy is transitory, or whether it is likely to be a predictor of long-term gender differences in mental rotation ability. We know from meta-analytic studies (Linn & Petersen, 1985; Voyer et al., 1995) that the gender difference in mental rotation increases from childhood to adolescence and remains relatively stable in adulthood. In addition, one early study (Hier & Crowley, 1982) found that men with idiopathic hypogonadotropic hypogonadism, who experienced normal testosterone concentrations prenatally but low concentrations of testosterone after birth, including during mini-puberty, had reduced spatial abilities, whereas men with *acquired* hypogonadotropic hypogonadism, who experienced low concentrations of testosterone only after puberty, had normal spatial abilities. This finding suggests that the influence of androgens on spatial abilities is exerted postnatally and before puberty. Given that mini-puberty is the only period of increased testosterone concentrations

postnatally and before puberty (the rest of childhood is characterized by comparatively low concentrations of testosterone in both boys and girls), this finding also suggests that the early surge of testosterone during mini-puberty has lasting organizational effects on spatial performance.

Regarding our finding of a negative correlation between parents' disapproval of other-gender characteristics and girls' mental rotation abilities, the effects of this in later life are also difficult to assess at this stage. One recent study suggests that mothers' disapproval of other-gender characteristics correlates negatively with daughters' rejection of traditional gender roles and with career aspirations in adult life (Colaner & Rittenour, 2015). While it could be hypothesized, on this basis, that parental attitudes to gender could exert further influences on offspring's behavior, the specific effects on mental rotation abilities in later life are yet to be investigated.

Many other social and environmental factors also influence mental rotation ability. Object manipulation and experience in infancy (Möhring & Frick, 2013; Schwarzer et al., 2013b), play with manipulative toys, training in spatial tasks or in geometry and mathematics (Baenninger & Newcombe, 1989), engagement with computer games (Cherney, 2008; De Lisi & Wolford, 2002; Feng et al., 2007; Terlecki & Newcombe, 2005), and adherence to masculine gender roles (Reilly & Neumann, 2013) have all been found to correlate positively with improved mental rotation performance. Therefore, the most reasonable conjecture at this stage is that testosterone and parents' attitudes to gender during early infancy are two among the many predictors of mental rotation ability later in life.

## **Conclusion**

The present study investigated the relationship of early postnatal testosterone concentrations and of parental gender-related attitudes to mental rotation performance in early infancy. Male infants were found to perform better than female infants on a 3D mental rotation task at 5 – 6 months of age. In addition, salivary testosterone concentrations were found to be higher in male than in female infants at 1 – 2.5 months of age, but not at 5 – 6 months of age. In boys, concentrations of salivary testosterone at age 1 – 2.5 months correlated positively with subsequent mental rotation performance at age 5 – 6 months. These results suggest that testosterone may have organizational influences on mental rotation performance. In girls, mental rotation performance at age 5 – 6 months correlated negatively with parents' traditional attitudes on gender. This finding suggests that parents could influence their daughters' mental rotation abilities beginning very early in life.

## Acknowledgements

We thank all the families who made this study possible through their participation.

### Figure Legends

- *Fig. 1.* Simplified Shepard-Metzler objects (from Moore & Johnson, 2008). The object shown on the left is arbitrarily called the L-object, and its mirror image, shown on the right, is arbitrarily called the R-object.
- *Fig. 2.* Still frames showing different rotation angles of the stimuli, as presented in the habituation and test videos. In this habituation video, the L-object moved back and forth through a nearly 240° arc. The test video then presented either the familiar L-object or its mirror image (the R-object), rotating back and forth through a previously unseen nearly 120° arc.
- *Fig. 3.* Salivary testosterone (pg/ml) at 1 – 2.5 months (individual and mean values).
- *Fig. 4.* Salivary testosterone (pg/ml) at 5 – 6 months (individual and mean values).
- *Fig. 5.* Five to six-months-old infants' looking times (in s) at the novel and familiar stimuli.
- *Fig. 6.* Testosterone concentrations (age 1 – 2.5 months) and mental rotation performance (age 5 – 6 months) in boys.
- *Fig. 7.* Testosterone concentrations (age 1 – 2.5 months) and mental rotation performance (age 5 – 6 months) in girls.
- *Fig. 8.* Disapproval of other-gender characteristics subscale and mental rotation performance in boys.
- *Fig. 9.* Disapproval of other-gender characteristics subscale and mental rotation performance in girls.

*Table 1.* Correlations between mental rotation scores and Child Gender Socialization Scale scores in boys and girls \*\* Correlation is significant at the 0.01 level (2-tailed)

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